

Remarks

Upon entry of the forgoing amendment, claims 31-70 are pending in the application, with claims 31, 33, 35, 36, 48, 60, 61, and 70 being the independent claims.

New claims 61-70 are sought to be added. Support for new claims 61-70 may be found throughout the specification and Figures. For example, support for claims 61-63 may be found on p. 11, lns. 13-28; p. 15, lns. 15-16; p. 19, lns. 24-30; p. 21, lns. 7-16, p. 24, ln. 24 to p. 30, ln. 23; p. 32, lns. 10-20; p. 34, lns 15-21; p. 58, ln. 1 to p. 60, ln. 28 and Figures 1A, 1B, 5A-5D, 6A-6D, and 7A-7D. Support for claim 64 may be found *e.g.*, in the passages listed above, along with at p. 43, ln. 14. Support for claims 65-68 may be found, *e.g.*, in the passages listed above, along with at p. 42, ln. 28 to p. 43, ln. 6. Support for claim 69 may be found, *e.g.*, in the passages listed above, along with at p. 43, lns. 19-20. Support for claim 70 may be found, *e.g.*, in the passages listed above, along with at p. 35, lns. 12-19; p. 45, ln. 25 to p. 46, ln. 13; p. 47, ln. 20 to p. 48, ln. 6; p. 48, lns. 18 to p. 49, ln. 26; and p. 50, lns. 1-8.

The addition of the new claims is believed to introduce no new matter. Applicants respectfully request that this Preliminary Amendment be entered by the Examiner.

For the Examiner's convenience, a copy of all of the pending claims, including those in the present Preliminary Amendment, are attached as Appendix A.

Respectfully submitted,

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Version with markings to show changes made

Claims 61-70 have been added.



Appendix A

Pending Claims:

31. A method for determining at least one previously unidentified biological function of a target protein comprising:

- (a) screening a multiplicity of different molecules for their ability to modify the stability of a target protein, wherein modification of the stability of said target protein by a molecule indicates that the molecule binds to said target protein;
- (b) generating, from step (a), a first list of molecules that modify the stability of said target protein;
- (c) comparing said first list from step (b) to at least one second list of molecules, wherein said second list of molecules are known to modify the stability of a group of proteins which share biological function; and
- (d) determining if any molecule in said first list from step (b) is included in said second list from step (c), thereby determining at least one previously unidentified biological function of said target protein.

32. The method of claim 31, wherein said screening step (a) comprises:

- (a1) contacting said target protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;
- (a2) treating said target protein in each of said multiplicity of containers to cause said target protein to unfold;
- (a3) measuring in each of said containers a physical change associated with the unfolding of said target protein;
- (a4) generating an unfolding curve for said target protein for each of said containers; and
- (a5) comparing each of said unfolding curves in step (a4) to (1) each of said other unfolding curves and to (2) the unfolding curve obtained for said target protein in the absence of any of said multiplicity of different molecules; and

(a6) determining whether any of said multiplicity of different molecules modifies the stability of said target protein, wherein a modification in stability is indicated by a change in said unfolding curve.

33. A method for determining at least one previously unidentified biological function of a target protein comprising:

(a) screening a multiplicity of different molecules from a first list of molecules for their ability to modify the stability of a target protein, wherein said first list of molecules are known to modify the stability of a group of proteins which share biological function, and wherein modification of the stability of said target protein by a molecule indicates that the molecule binds to said target protein:

(b) generating, from step (a), a second list of molecules that modify the stability of said target protein:

(c) determining if any molecule in said first list from step (a) is included in said second list from step (b), thereby determining at least one previously unidentified biological function of said target protein.

34. The method of claim 33, wherein said screening step (a) comprises:

(a1) contacting said target protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) treating said target protein in each of said multiplicity of containers to cause said target protein to unfold:

(a3) measuring in each of said containers a physical change associated with the unfolding of said target protein:

(a4) generating an unfolding curve for said target protein for each of said containers; and

(a5) comparing each of said unfolding curves in step (a4) to (1) each of said other unfolding curves and to (2) the unfolding curve obtained for said target protein in the absence of any of said multiplicity of different molecules; and

(a6) determining whether any of said multiplicity of different molecules modifies the stability of said target protein, wherein a modification in stability is indicated by a change in said unfolding curve.

35. A method for determining at least one previously unidentified biological function of a target protein comprising:

determining at least one previously unidentified biological function of said target protein if molecules that modify the stability of said target protein modify the stability of proteins which share biological function.

36. A method for determining at least one previously unidentified biological function of a target protein comprising:

(a) screening a multiplicity of different molecules for their ability to shift the thermal unfolding curve of a target protein, wherein a shift in the thermal unfolding curve of said target protein by a molecule indicates that the molecule binds to said target protein;

(b) generating, from step (a), a first list of molecules that shift the thermal unfolding curve of said target protein;

(c) comparing said first list from step (b) to at least one second list of molecules, wherein said second list of molecules are known to modify the stability of a group of proteins which share biological function; and

(d) determining if any molecule in said first list from step (b) is included in said second list from step (c), thereby determining at least one previously unidentified biological function of said target protein.

37. The method of claim 36, wherein said screening step (a) comprises:

(a1) contacting said protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) heating said multiplicity of containers from step (a1);

- (a3) measuring in each of said containers a physical change associated with the thermal unfolding of said target protein resulting from said heating;
- (a4) generating a thermal unfolding curve for said target protein as a function of temperature for each of said containers; and
- (a5) comparing each of said unfolding curves in step (a4) to (1) each of said other thermal unfolding curves and to (2) the thermal unfolding curve obtained for said protein in the absence of any of said multiplicity of different molecules; and
- (a6) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said protein.

38. The method of claim 37, wherein said comparing step (a5) comprises ranking said molecules in said multiplicity of different molecules for binding to said target protein according to the ability of each of said multiplicity of different molecules to shift the thermal unfolding curve of said target protein.

39. The method of claim 37, wherein in said heating step (a2), said multiplicity of containers is heated simultaneously.

40. The method of claim 37, wherein said step (a4) further comprises determining a midpoint temperature (T_m) from the thermal unfolding curve; and wherein said step (a5) further comprises comparing the T_m of each of said unfolding curves in step (a4) to (1) the T_m of each of said other thermal unfolding curves and to (2) the T_m of the thermal unfolding curve obtained for said target protein in the absence of any of said different molecules.

41. The method of claim 37, wherein said step (a3) comprises measuring the absorbance of light by said contents of each of said containers.

42. The method of claim 37, wherein said step (a1) comprises contacting said target protein with a fluorescence probe molecule present in each of said multiplicity of containers and wherein said step (a3) comprises

- (i) exciting said fluorescence probe molecule, in each of said multiplicity of containers, with light; and
- (ii) measuring the fluorescence from each of said multiplicity of containers.

43. The method of claim 42, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers one container at a time.

44. The method of claim 42, wherein said step (a3)(ii) further comprises measuring the fluorescence from a subset of said multiplicity of containers simultaneously.

45. The method of claim 42, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers simultaneously.

46. The method of claim 37, wherein said step (a3) comprises

- (i) exciting tryptophan residues in said target protein, in each of said multiplicity of containers, with light; and
- (ii) measuring the fluorescence from each of said multiplicity of containers.

47. The method of claim 37, wherein said multiplicity of containers in step (a1) comprises a multiplicity of wells in a microplate.

48. A method for determining at least one previously unidentified biological function of a target protein comprising:

- (a) screening a multiplicity of different molecules from a first list of molecules for their ability to shift the thermal unfolding curve of a target protein.

wherein said first list of molecules are known to modify the stability of a group of proteins which share biological function, and wherein a shift in the thermal unfolding curve of said target protein by a molecule indicates that the molecule binds to said target protein;

(b) generating, from step (a), a second list of molecules that modify the stability of said target protein;

(c) determining if any molecule in said first list from step (a) is included in said second list from step (b), thereby determining at least one previously unidentified biological function of said target protein.

49. The method of claim 48, wherein said screening step (a) comprises:

(a1) contacting said protein with one or more of said multiplicity of different molecules in each of a multiplicity of containers;

(a2) heating said multiplicity of containers from step (a1);

(a3) measuring in each of said containers a physical change associated with the thermal unfolding of said target protein resulting from said heating;

(a4) generating a thermal unfolding curve for said target protein as a function of temperature for each of said containers; and

(a5) comparing each of said unfolding curves in step (a4) to (1) each of said other thermal unfolding curves and to (2) the thermal unfolding curve obtained for said protein in the absence of any of said multiplicity of different molecules; and

(a6) determining whether any of said multiplicity of different molecules shift the thermal unfolding curve of said protein.

50. The method of claim 49, wherein said comparing step (a5) comprises ranking said molecules in said multiplicity of different molecules for binding to said target protein according to the ability of each of said multiplicity of different molecules to shift the thermal unfolding curve of said target protein.

51. The method of claim 49, wherein in said heating step (a2), said multiplicity of containers is heated simultaneously.

52. The method of claim 49, wherein said step (a4) further comprises determining a midpoint temperature (T_m) from the thermal unfolding curve; and wherein said step (a5) further comprises comparing the T_m of each of said unfolding curves in step (a4) to (1) the T_m of each of said other thermal unfolding curves and to (2) the T_m of the thermal unfolding curve obtained for said target protein in the absence of any of said different molecules.

53. The method of claim 49, wherein said step (a3) comprises measuring the absorbance of light by said contents of each of said containers.

54. The method of claim 49, wherein said step (a1) comprises contacting said target protein with a fluorescence probe molecule present in each of said multiplicity of containers and wherein said step (a3) comprises

- (i) exciting said fluorescence probe molecule, in each of said multiplicity of containers, with light; and
- (ii) measuring the fluorescence from each of said multiplicity of containers.

55. The method of claim 54, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers one container at a time.

56. The method of claim 54, wherein said step (a3)(ii) further comprises measuring the fluorescence from a subset of said multiplicity of containers simultaneously.

57. The method of claim 54, wherein said step (a3)(ii) further comprises measuring the fluorescence from each of said multiplicity of containers simultaneously.

58. The method of claim 49, wherein said step (a3) comprises
- (i) exciting tryptophan residues in said target protein, in each of said multiplicity of containers, with light; and
 - (ii) measuring the fluorescence from each of said multiplicity of containers.
59. The method of claim 48, wherein said multiplicity of containers in step (a1) comprises a multiplicity of wells in a microplate.
60. A method for determining at least one previously unidentified biological function of a target protein comprising:
determining at least one previously unidentified biological function of said target protein if molecules that shift the thermal unfolding curve of said target protein shift the thermal unfolding curves of proteins which share biological function.
61. A functional probe library for determining at least one previously unidentified biological function of a target protein, comprising
a multiplicity of discrete compounds, said multiplicity made up of a plurality of vitamins, a plurality of coenzymes, a plurality of compounds having amino acid residue functional groups and mimics thereof, a plurality of metal chelators, a plurality of metal ions, a plurality of carbohydrates, a plurality of nucleic acids, a plurality of lipids, a plurality of enzymes, a plurality of steroids, a plurality of amine hormones, a plurality of alkaloids, a plurality of generic drug molecules and a plurality of natural products;
wherein said library has a sufficient diversity of compounds to determine at least one previously unidentified biological function of the target protein when the multiplicity of compounds of the functional probe library are tested for their ability to modify the stability of the target protein and are compared to a list of compounds known to modify the stability of a group of proteins which share biological function.
62. The functional probe library of claim 61, wherein one or more of said compounds are provided in separate wells of a microtiter plate.

63. The functional probe library of claim 62, wherein each discrete compound is provided singly in a microtiter plate well.
64. The functional probe library of claim 62, wherein said separate wells of said microtiter plate each contain from 1 μ L to 100 μ L of total solution.
65. The functional probe library of claim 62, wherein said microtiter plate has 96 wells.
66. The functional probe library of claim 62, wherein said microtiter plate has 384 wells.
67. The functional probe library of claim 62, wherein said microtiter plate has 864 wells.
68. The functional probe library of claim 62, wherein said microtiter plate has 1536 wells.
69. The functional probe library of claim 62, wherein said microtiter plate is a polypropylene plate.
70. An apparatus for determining at least one previously unidentified biological function of a target protein comprising:
a first heat conducting block in contact with a first plurality of samples provided in separate wells of a microtiter plate, each of said samples comprising said target protein and one or more of a multiplicity of discrete compounds from a functional probe library;
a temperature controller coupled to said first heat conducting block;
a light source disposed adjacent to said first heat conducting block;

a fluorescence emission sensor disposed adjacent to said first heat conducting block; and

means for processing a spectral emission signal obtained from said fluorescence emission sensor;

wherein the multiplicity of discrete compounds from the functional probe library is made up of a plurality of vitamins, a plurality of coenzymes, a plurality of compounds having amino acid residue functional groups and mimics thereof, a plurality of metal chelators, a plurality of metal ions, a plurality of carbohydrates, a plurality of nucleic acids, a plurality of lipids, a plurality of enzymes, a plurality of steroids, a plurality of amine hormones, a plurality of alkaloids, a plurality of generic drug molecules and a plurality of natural products; and

wherein said functional probe library has a sufficient diversity of compounds to determine at least one previously unidentified biological function of the target protein when the multiplicity of compounds from the functional probe library are tested using said apparatus for their ability to shift the thermal unfolding curve of the target protein and are compared to a list of compounds known to modify the stability of a group of proteins which share biological function.